PATENT
Attorney Docket No.: CHEM1100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	J. Leng	Art Unit:	1642
Application No.:	09/559,874	Examiner	Rawlings, S.
Filed:	April 25, 2000		
Title:	CELL PROLIFERATION ASSAY		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF INVENTOR JAY LENG

Sir:

As a below-named inventor, I hereby declare that:

Considered SR 1/14/04

1. I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: "CELL PROLIFERATION ASSAY," Application Serial No. 09/559,874 ("The '874 application"). I am familiar with prosecution of the '874 application.

2. I have performed experiments, as set forth in Exhibit A, to confirm the correlation between cell viability and bioluminescence, as set forth in the teachings of the '874 application.

3. The experiment shown in Exhibit A provides an assay whereby two cell concentrations (12500 cells/well and 195 cells/well) are measured for cell proliferation with and without Cytochalasin D, an inhibitor of cell proliferation. Two different assays were utilized, an MTS assay, a commonly accepted method of assaying for cell proliferation, and a Luciferase Assay, according to the teachings of the '874 application.

4. The results of the assays set forth in Exhibit A demonstrate that the luciferase assay, as described in the teachings of the '874 application, when compared to the results of an MTS assay, demonstrate the ability of the luciferase assay to measure cell proliferation. As compared to the MTS assay, the luciferase assay showed cell proliferation in the absence of Cytochalasin D and inhibition of proliferation in the presence of Cytochalasin D, at both cell concentration levels by measuring bioluminescence.

5. The results of the experiments set forth in Exhibit A confirm the existence of a correlation between cell viability and bioluminescence.

CERTIFICATION UNDER 37 CFR §1.6(d)

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below

Karen LePari
(Name of person transmitting paper)

Karen LePari 7-14-03
(Signature) *(Date)*

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 7-14-2003

Jay Leng

Jay Leng

EXHIBIT A

Comparison of MTS and Luciferase assays in the presence and absence of Cytochalasin D

Cell Culture

- HT-1080 R. Luciferase stable cells were seeded at 2 concentrations (12000 cells/well and 195 cells/well) in 100uL of DMEM + 10% FCS for 4 hours @ 37° C.
- 50 uL of DMEM + 10% FCS (+/- 3ug/mL Cytochalasin D) was added to the wells and incubated for 24 hours @ 37° C.

MTS detection

1. 20uL of MTS solution was added to each well and incubated for 1.5 hours @ 37° C.
2. Read plate at 490nm.

Results:

MTS Assay (OD490)					
	- Cyto D		+ Cyto D		
Concentration (Cell #/well)	Raw Data	Raw Data - background	Raw Data	Raw Data - background	% Inhibition
12500	0.782	0.479	0.450	0.144	69.94
195	0.323	0.020	0.311	0.005	75.00
0 (background)	0.303		0.306		

Figure 1: Bar graph of cell proliferation results of MTS assay at concentrations of 12500 cells/well and 195 cells/well.

Luciferase detection

1. Media was aspirated from the wells
2. Cells were lysed with 25uL of 1X Passive Lysis Buffer for 15 minutes @ 25° C.
3. Transferred the solution to a luminometer plate
4. Read luminescence (150uL injection with 15 sec integration)

Results:

Luciferase Assay (RLU)					
	- Cyto D		+ Cyto D		
Concentration (Cell #/well)	Raw Data	Raw Data - background	Raw Data	Raw Data - background	% Inhibition
12500	312.5	311.989	120.4	120.183	61.48
195	7.469	6.958	2.153	1.936	72.18

Luciferase Assay (RLU)					
	- Cyto D		+ Cyto D		
Concentration (Cell #/well)	Raw Data	Raw Data - background	Raw Data	Raw Data - background	**% Inhibition**
0 (background)	0.511		0.217		

Figure 2: Bar graph of cell proliferation results of Luciferase assay at concentrations of 12500 cells/well and 195 cells/well.

Figure 3: Comparison of percent inhibition detected by MTS assay versus percent inhibition detected by Luciferase assay at concentrations of 12500 cells/well and 195 cells/well.

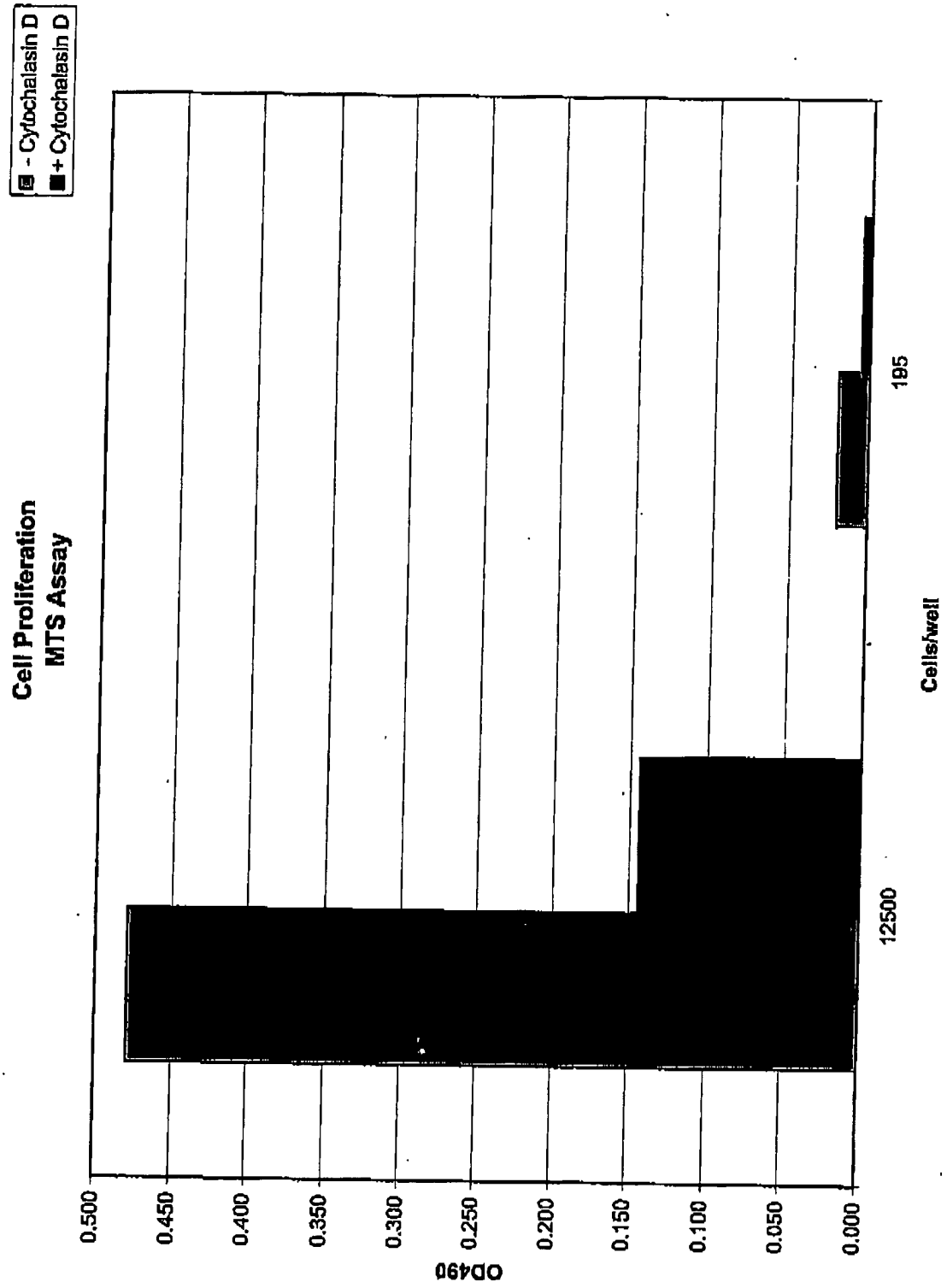

FIGURE 1

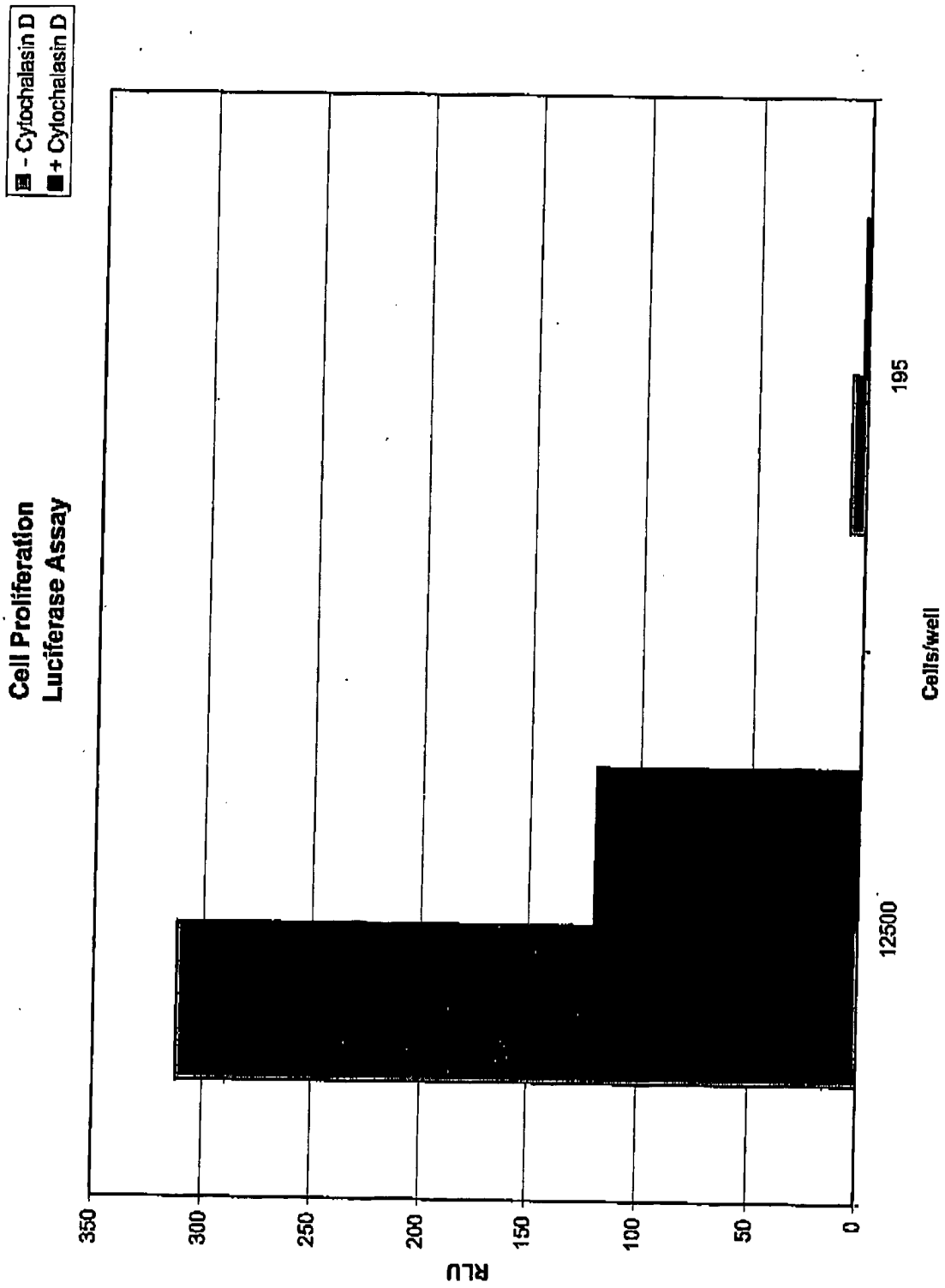

FIGURE 2

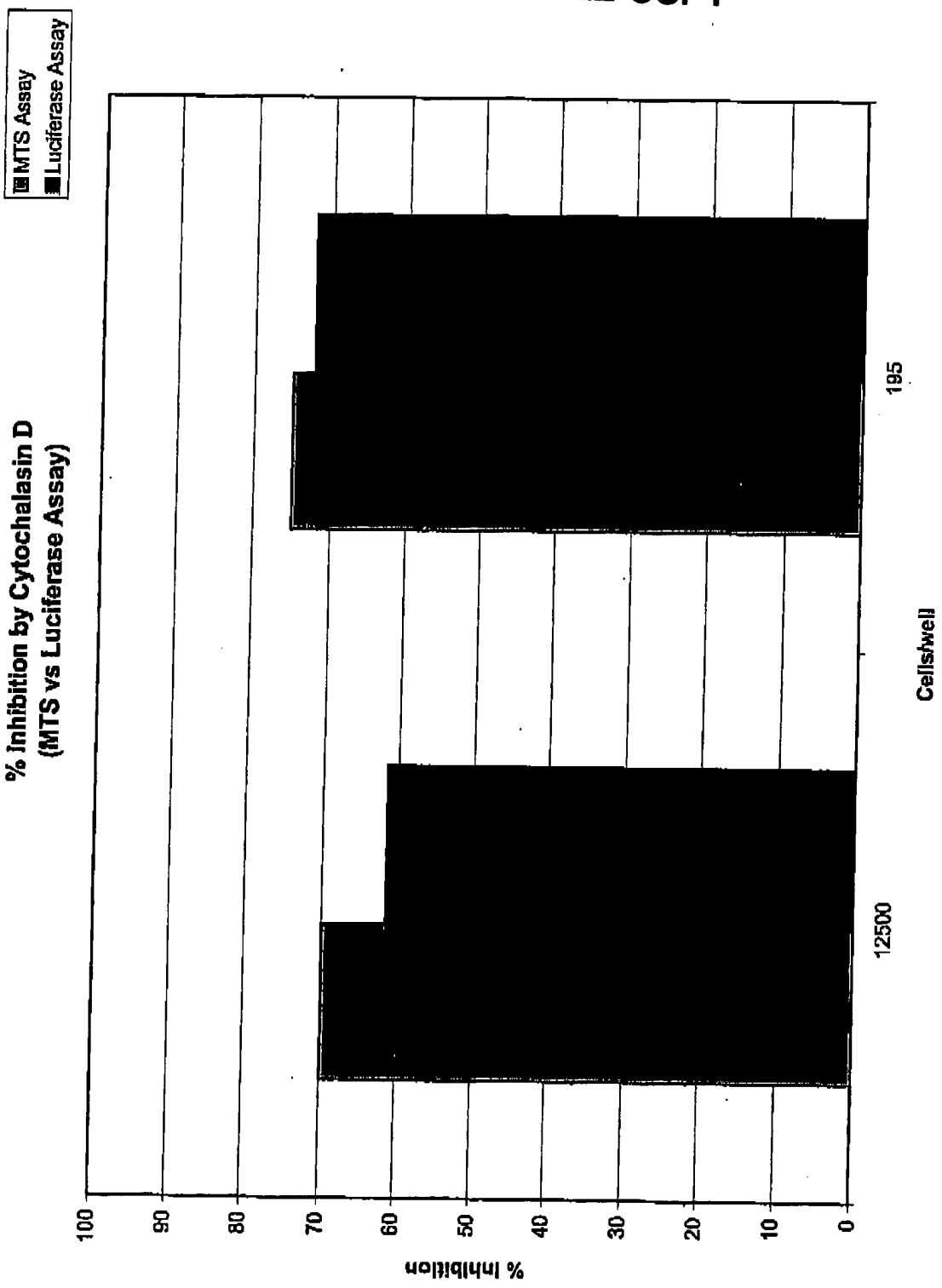

FIGURE 3